Protein and Sugar Separation by Mechanochemical Membrane Having "Chemical Valve" Function[†]

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ABSTRACT: The membrane permeability of aqueous solutions of proteins (albumin, hemoglobin, amylase, invertase), sugars (glucose, raffinose), and their mixtures was investigated for poly(methacrylic acid) membranes treated and untreated with poly(ethylene glycol) (PEG). It was found that the PEG-treated membranes allow a much faster permeation of these macro- and microsolutes as well as water than untreated ones, but that solute rejection depends largely on the molecular weight of the solute substance. Enhanced permeation of fluid through PEG-treated membranes is caused by mechanochemical contraction of the network chains which tend to expand the micropores of the membrane.

KEY WORDS   Mechanochemical Reaction / Ultrafiltration / Protein Separation / Polymer Complexation / Poly(methacrylic acid) / Poly(ethylene glycol) /

Recently, we found a mechanochemical membrane which acts as a "chemical valve."[1] This membrane consists of a three-dimensional network of poly(methacrylic acid) (PMAA) and its micropores reversibly expand by treatment with an aqueous solution of poly(ethylene glycol) (PEG). The expansion of pores is brought about isothermally as a result of contraction of the network chains that occurs when PMAA forms a reversible complex with PEG. The details of the complex formation and the accompanying energy transformation are discussed elsewhere.[2-5]

Our previous experiment demonstrated that PEG-treated membranes of PMAA allow a rapid permeation of aqueous solutions of some proteins. We performed a similar experiment with other proteins, sugars, and their mixtures, hoping to obtain more information on the permeation behavior of these membranes. The results are presented in this paper.

EXPERIMENTAL

A PMAA sample having a molecular weight of 7.8×10^5 was used for the present work. Membranes were prepared by the method described previously.[2,4] 100 ml of an aqueous solution containing 3.3 g of the polymer sample and 15 ml of glycerol was cast on a glass plate and heated for 24 h at 120°C. When brought to equilibrium in water at room temperature, the membrane so prepared was 30 μm thick, contained 90—150% water relative to the weight of dry membrane, and increased in length by *ca.* 20%.

The solutes examined were albumin (human, Tokyo Kasei), hemoglobin (from beef blood, Sigma Chemical), α-amylase (Type IIIA, Nakarai Chemical), invertase (from yeast, Wako Chemical), D-(+)-raffinose (Nakarai Chemical), and D-glucose (Kanto Chemical). All were used as delivered.

Hydraulic permeability measurements were carried out using the commercial ultrafiltration apparatus (Bioengineering Co., MC-1 Type) illustrated in Figure 1. The cell volume was 10 ml and

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the effective membrane area was 3.4 cm². The cell was immersed in a water bath maintained at 30°C, and a pressure of 0.1—0.2 kg cm⁻² was applied to the liquid in the cell with compressed argon.

Each flow test was performed as follows. First, depending on the water permeability of a given untreated membrane, 0.1—5 ml of water was allowed to permeate through the membrane. The water in the cell was then replaced by 10 ml of an aqueous solution of PEG (molecular weight = 20000, concentration = 0.058 mol l⁻¹). After some of the PEG permeated through the membrane, the remaining solution was removed from the cell and the membrane was rinsed thoroughly with water. 10 ml of an aqueous solution of the test substance was then placed in the cell, and the solute concentrations C_f and C_p in the feed and permeated solutions were monitored as a function of time, either polarimetrically with a JASCO DIP 140 polarimeter or spectroscopically with a Hitachi 200-100 spectrophotometer. Also, during this series of operations, the volume of permeated fluid was measured using a
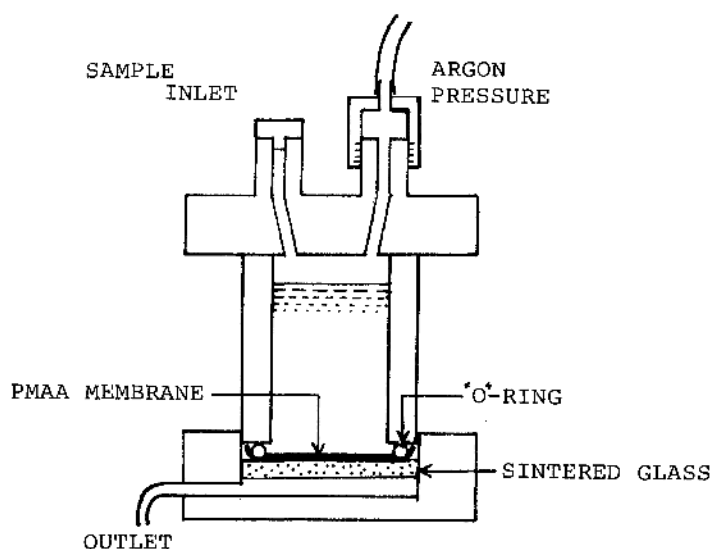

Figure 1. Apparatus for flow tests.

**Table I.** Results of flow tests on proteins with PMAA membranes treated and untreated with PEG

| | Albumin | | Hemoglobin | | Amylase | | Amylase + Albumin | | | |
| | | | | | | | Untreated | | Treated | |
	Untreated	Treated	Untreated	Treated	Untreated	Treated	Amylase	Albumin	Amylase	Albumin
F_0 (×10⁻⁵)ᵃ	4.7	1.9	4.7	27	46	38	127		13.2	
F (×10⁻⁵)ᵃ	0.51	57	0.32	150	45	106	4.2		2.9	
F/F_0	0.11	31	0.07	5.2	0.97	2.8	0.03		0.22	
R	0.51	0.43	0.78	0.53	0.035	0.035	0.06	0.38	0.06	1.00
V (×10⁻²)	5.5	1300	5.2	290	3.4	9.8	0.2	1.25	1.4	21.9

ᵃ (cm³ s⁻¹ cm⁻²); F_0 for PEG-treated membranes were measured prior to treatment with PEG.

Table II. Results of flow tests on sugars with PMAA membranes
treated and untreated with PEG

| | Raffinose | | Glucose + Raffinose | | | |
| | | | Untreated | | Treated | |
	Untreated	Treated	Glucose	Raffinose	Glucose	Raffinose
F_0 ($\times 10^{-5}$)[a]	395	39	347		72.1	
F ($\times 10^{-5}$)[a]	15	59	0.98		2.9	
F/F_0	0.04	1.52	0.003		0.041	
R	0.02	0.02	0.18	0.30	0.23	0.34
V ($\times 10^{-3}$)	0.76	31	0.52	0.85	9.3	14

[a] ($cm^3 s^{-1} cm^{-2}$); F_0 for PEG-treated membranes were measured prior to treatment with PEG.
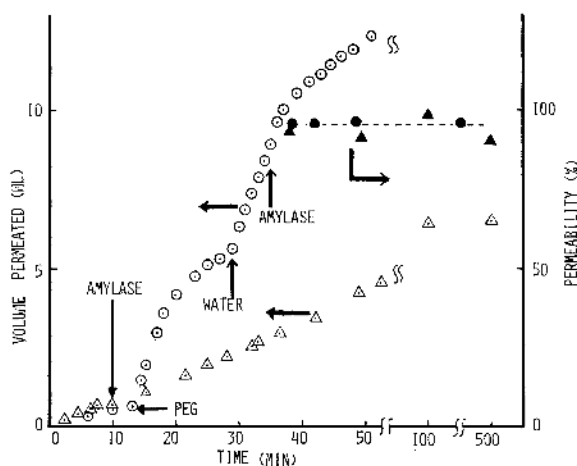
Figure 2. Time changes in the volume of permeated aqueous amylase and solute permeability (in percent). Circles, PEG-treated PMAA membrane; triangles, untreated PMAA membrane. Experimental conditions: amylase, 0.025 wt%; pressure, 0.2 kg cm^{-2}.
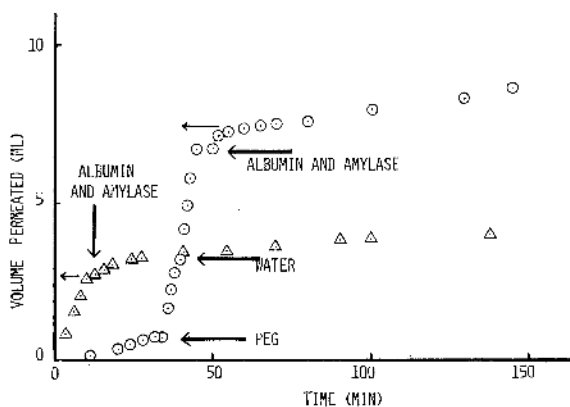
Figure 3. Time changes in the volume of permeated aqueous mixture of amylase and albumin. Symbols are the same as those in Figure 2. Experimental conditions: amylase, 0.025 wt%; albumin, 0.025 wt%; pressure, 0.1 kg cm^{-2}.
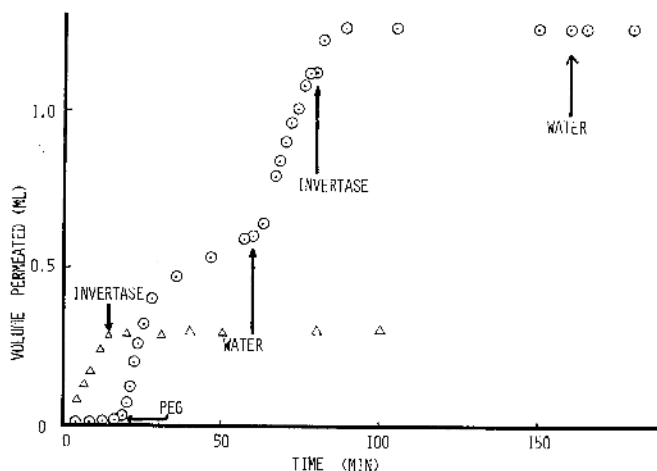
Figure 4. Time changes in the volume of permeated aqueous invertase. Symbols are the same as those in Figure 2. Experimental conditions: invertase, 0.025 wt%; pressure, 0.2 kg cm^{-2}.

graduated cylinder at suitable intervals of time to obtain a permeation curve.

RESULTS AND DISCUSSION

Numerical data of all the flow tests made in this work are summarized in Tables I and II. In these, R is the solute rejection defined by $1 - P$, with $P = C_p/C_f$ (P is called the solute permeability), and V is the separation performance defined by RF/F_0, with F_0 and F being the fluxes of water and a solution, respectively. F_0 and F were determined from the slopes of the linear portion of the permeation curves assuming that the stationary flow state was attained in this portion.

Typical observed results are shown graphically in Figures 2 through 6. In these, the circles refer to a PEG-treated PMAA membrane and the triangles to an untreated membrane, and the arrows, either vertical or horizontal, indicate the times at which water or a solution (of PEG or the solute concerned) was added to the cell.

In all cases studied, the PEG-treated membrane allowed a faster permeation of fluid than the untreated membrane, as can be seen from these figures. This finding indicates that the micropores of a PMAA membrane are certainly widened by the interaction of PMAA with PEG which induces the contraction of the PMAA network.

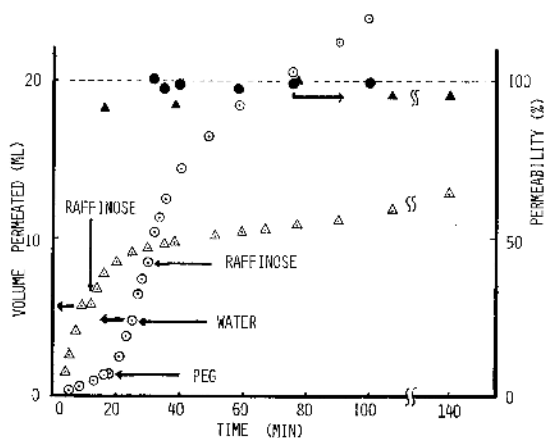Figure 2 shows that P for amylase is essentially independent of whether the membrane is treated

Figure 5. Time changes in the volume of permeated aqueous raffinose and solute permeability (in percent). Symbols are the same as those in Figure 2. Experimental conditions: raffinose, 9.4 wt%; pressure, 0.2 kg cm^{-2}

with PEG or not and equals about 96%. This finding may be attributed to the relatively small radius of the amylase molecule. In fact, our previous study[1] showed (also see Table 1 of this paper) that the P values for albumin and hemoglobin, which have larger molecular radii than amylase (molecular weight: albumin = 67000; hemoglobin = 64000; amylase = 45000), were about 50% or less for both PEG-treated and untreated membranes.

The data for a mixture of amylase and albumin in Table I indicate that the R value for amylase was
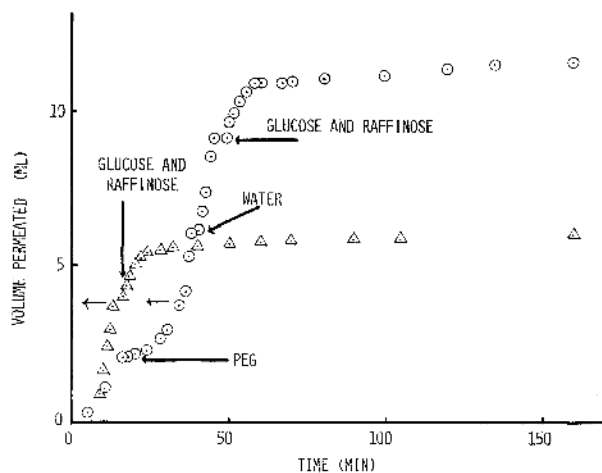
Figure 6. Time changes in the volume of permeated aqueous mixture of raffinose and glucose. Symbols are the same as those in Figure 2. Experimental conditions: raffinose, 5.0 wt%; glucose, 5.0 wt%; pressure, 0.1 kg cm^{-2}.
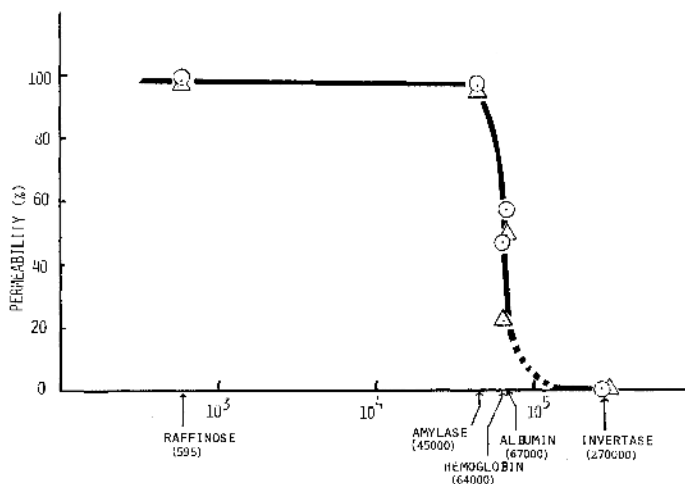
Figure 7. Dependence of solute permeability on molecular weight of the solute substance. Symbols are the same as those in Figure 2.

quite small (0.06) for both PEG-treated and untreated PMAA membranes, but that for albumin, increased from 0.38 to 1.00 when an original PMAA membrane was treated with PEG. This finding is very interesting, since it implies that almost complete separation of these two proteins from their mixture can be achieved by use of a PMAA membrane treated with PEG.

The membranes, both PEG-treated and untreated, used for flow tests with invertase (molecular weight $= 27 \times 10^4$) allowed practically no per-

meation of the protein as well as water in spite of the fact that the membrane treated with PEG increased the rate of water permeation by about 50 times (Figure 4).

The flow test data shown in Figure 5 indicate that the P value for a trisaccharide raffinose is essentially unity (see also Table II) for both PEG-treated and untreated membranes, i.e., these membranes are not capable of rejecting raffinose from its aqueous solution. This is probably due to the small molecular size of the solute.

According to the data for a mixture of glucose and raffinose in Table II, the separation performance for mono- and trisaccharides increased considerably as a result of their coexistence in the solution, but no effective separation of the two solutes was achieved even by a membrane treated with PEG, in contrast to the case of amylase + albumin mixture. This is probably due to the fact that these saccharides are too small in molecular size.

In Figure 7, the P values for the solutes studied are plotted against molecular weight. The data points are approximately fitted by a single curve. It can be seen that, regardless of whether expanded by the treatment with PEG or not, the micropores of a PMAA membrane essentially "cut off" macrosolutes of molecular weights higher than about 70000.

The findings reported in this paper suggest the usefulness of our mechanochemical PMAA membrane for the ultrafiltration of biological solutes.

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